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# Characterization of Planktonic and Biofilm Communities of Day-of-Hatch Chicks Cecal Microflora and Their Resistance to *Salmonella* Colonization†

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## ABSTRACT

Recent concerns about the use of antimicrobials in food animals have increased interest in the microbial ecology and biofilms within their gastrointestinal tract. This work used a continuous-flow chemostat system to model the microbial community within the ceca from day-of-hatch chicks and its ability to resist colonization by *Salmonella enterica* serovar Typhimurium. We characterized the biofilm and planktonic communities from five cultures by using automated ribotyping. Eight species from six different genera were identified. Overall, the planktonic communities were more diverse, with 40% of the cultures containing four or more bacterial species. Eighty percent of the biofilm communities contained only one or two species of bacteria. *Enterococcus faecalis* was the only species isolated from all communities. None of the resulting microbial communities was able to resist colonization by *S. enterica* serovar Typhimurium. This is the first study to provide a molecular-based characterization of the biofilm and planktonic communities found in day-of-hatch chicken cecal microflora cultures.

Until the 1950s, all poultry production in the United States was "free range," in that all poultry was raised outdoors and not segregated by age. When producers began using indoor, confinement production, they gained better protection from predators and tighter control of operations, and increased their efficiency and overall production. However, producers lost the benefits of the protection provided to the newly hatched chicks from the natural exposure to adult protective microbial communities via contact with adults and their feces *(14)*. Thus, an understanding of the microbial ecology of the poultry gastrointestinal tract (GIT) can aid poultry producers in improving their management practices and increasing overall profitability and consumer safety.

Freter et al. *(15)* were among the first researchers to study gastrointestinal bacterial interactions by using a continuous-flow chemostat. The continuous-flow culture system is an in vitro model for the study of microbes in a controlled and nearly constant environment in which growth conditions are maintained for microorganisms over extended periods, by supplying a continuous renewal of nutrients. It provides an experimental environment in which the gastrointestinal system can be simulated under controlled laboratory conditions to allow investigation of the community dynamics of microflora *(16, 17, 32)*. Often a stabilized culture has two components, (i) free-floating microorganisms in the liquid medium—the planktonic component, and (ii) surface-attached, aggregated microorganisms—the biofilm component. The continuous-flow chemostat system provides researchers with a tool to elucidate relationships between antibiotics and bacteria *(9, 35)*, and to develop effective competitive exclusion cultures *(21, 31, 41, 50)*. However, there is a lack of information concerning the characterization of biofilms and their influence on the development of probiotics within planktonic portion of a culture.

Bower and Daeschel *(7)* demonstrated that bacterial biofilm formation on food processing surfaces served as an avenue for the development or enhancement of bacterial resistance. Spoering and Lewis *(44)* postulated that one of the survival advantages provided by biofilms is the existence of subpopulations of resistant phenotypes in the biofilms. These microorganisms were dubbed "persisters" and represent only a very small fraction of the entire biomass of a culture *(46)*. Biofilms are utilized by microbes as an important strategy for survival in both natural and engineered environments *(24)*. According to Hall-Stoodley et al. *(18)*, "The realization of the extent to which microbial growth and development occurs on surfaces in complex communities has been one of the most subtle advances in microbiology over the past 50 years." Researchers now recognize that biofilms are not just passive aggregations of cell adhering to surfaces, but are structurally complex and dynamic communities that represent substantial phenotypic diversification, and provide protection from a wide range of environmental challenges, such as UV exposure, metal toxicity, acid exposure, dehydration and salinity, phagocytosis, and several antibiotics and antimicrobial agents *(6, 18)*. Despite this interest in the role of biofilms on bacterial sur-

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vival and possibly the development and spread of bacterial antibiotic resistance, Wuertz et al. *(51)* point out that "the majority of biofilms studies only deal with adhesion, the first step of biofilms development, and that there are almost no microbial studies on biofilms older than one week."

An increased understanding of the factors affecting pathogen colonization in the GIT may help to provide improved control measures. Additionally, it has been shown by numerous researchers that the establishment of anaerobic populations within the GIT reduces or prevents the colonization of *Salmonella* in the cecum. This has been attributed to the production of inhibitory volatile fatty acids by the anaerobic organisms *(3, 13, 53)*. The cecum is the site of some of the most diverse microflora communities found within the GIT of poultry *(1, 3, 28)* and serves as an excellent source for the development of competitive exclusion cultures *(31, 36, 42)*. Ceca are a primary reservoir for *Salmonella* colonization in young chicks, as it can take more than 4 weeks for the ceca to establish a protective, native adult microfloral community *(22, 29, 35, 43)*. In this study, we used traditional bacteria isolations techniques combined with automated ribotyping to characterize both the planktonic and biofilm communities of continuous-flow cultures derived from the cecal microflora of day-of-hatch chicks, and evaluated the ability of these cultures to resist colonization by *Salmonella*.

## MATERIALS AND METHODS

**Chickens and cecal material collection.** German Lohmann Selected Leghorn layer chicks (Hy-Line International, Bryan, TX) were obtained from the hatchery on day of hatch, at which point they were 8 to 12 h old. Chicks were immediately euthanized, and the ceca were surgically removed under aseptic conditions. The Southern Plains Agricultural Research Center Animal Care and Use Committee approved all procedures. The cecal contents were collected, combined, and thoroughly mixed under sterile, anaerobic conditions. Aliquots of 1.2 ± 0.12 g of cecal material, each combined with modified Viande Levure (VL) medium (prepared in-house) *(33)*, were used to start five BioFlo I fermentors (New Brunswick Scientific Co., Edison, NJ) fitted with a 1-liter chemostat vessel. The first three cultures (A, B, and C) were each initiated from the combined cecal material from 5 chicks. In subsequent cultures (D and E), we increased the initial source material by combining the cecal contents of 10 chicks, and each culture was initiated from a replicate aliquots of this combined cecal material. The chemostat vessel was continuously flushed with a stream of $O_2$-free $CO_2$, and maintained a vessel turnover time of 24 h and a flow rate of 0.8 ml/min, as previously described *(33)*. Cultures were allowed to develop undisturbed for 3 weeks.

**Isolation procedures.** The following isolation procedures were performed under sterile aerobic and anaerobic conditions by using a series of selective media. Primary aerobic isolation of the planktonic community was performed by streaking a 10-μl aliquot of the culture onto plates, each containing one of the following media: MacConkey agar, m-*Enterococcus* agar, brilliant green agar, de Man Rogosa Sharpe agar (all from Fisher Scientific International, Inc., Hampton, NH), or chromogenic *E. coli*/coliform agar (Hardy Diagnostics, Santa Maria, CA). Plates were incubated for 24 h at 37°C. Over 300 isolates with both similar and different morphology were randomly selected and subcultured; purified isolates were screened for visual similarity, and duplicates were eliminated. The remaining isolates, over 158, were plated onto tryptic soy agar with 5% sheep blood and incubated aerobically for 24 h at 37°C. The resulting colony growth was used for ribotype analysis.

Primary anaerobic isolation of the planktonic community was performed by streaking a 10-μl aliquot of the culture onto plates each containing one of the following media: *Brucella* blood agar, *Bacteroides* bile esculin agar (both from Anaerobe Systems, Morgan Hill, CA), and *Veillonella* agar (Fisher Scientific International, Inc). Plates were incubated in a Bactron 1.5 anaerobic chamber (Sheldon Manufacturing, Cornelius, OR) for 48 to 72 h at 37°C, and isolates were randomly selected for subculture. Subculture purified isolates were then plated on tryptic soy agar with 5% sheep blood and incubated aerobically for 24 to 48 h at 37°C. Isolates found to be obligate anaerobes were plated on *Brucella* blood agar and incubated anaerobically for 48 to 72 h at 37°C. The resulting colony growth was used for ribotype analysis.

The bacterial diversity within the planktonic component of all chemostats was assessed after 3 weeks of culture. After this initial assessment, cultures A and D were challenged, as previously described, with sufficient inoculum to ensure a final bioreactor concentration of $10^6$ CFU/ml *(19, 23)* green fluorescent protein–expressing *Salmonella enterica* serovar Typhimurium (ST-GFP) *(20)*. The planktonic component of these chemostats was sampled for bacterial quantification prior to challenge and 15 min, 2 h, and 2, 4, 7, 9, 11, 14, 16, and 18 days postchallenge. Serial dilution of culture material onto brilliant green agar plates was performed in triplicate to determine bacterial concentration. Beginning on day 4, duplicate culture samples were enriched by incubation for 24 h at 37°C in tetrathionate broth, which was followed by plating on brilliant green agar and incubation for 24 h at 37°C *(2)*. When the 10-fold dilution plate counts for *Salmonella* were ≤10 CFU/ml, the related enriched sample was used to determine the presence or absence of *Salmonella (2)*. Chemostats B, C, and D were allowed to develop without challenge by ST-GFP for an additional 18 days. On day 39, the planktonic component was drained from each bioreactor. The bioreactor was flushed three times with anaerobic normal saline, and the biofilm community was collected in duplicate from each of five vessel levels within the chemostat by using two sterile cotton swabs per level: 1, bottom of the chemostat; 2, lowest area of the sidewall; 3, midway up the biofilm; 4, under the biofilm interface ring; and 5, biofilm interface ring. These levels were chosen in an effort to ensure that the biofilm communities analyzed were representative of the entire chemostat. After biofilm collection, swabs were immediately placed into anaerobic transport medium tubes (Anaerobe Systems). One swab was used to streak plates for anaerobic isolation, the other for aerobic isolation. Both anaerobic and aerobic isolation and culturing procedures followed the same scheme as described above.

In addition, three chemostat cultures containing VL media only were initiated and challenged on day 21 with ST-GFP and sampled prior to challenge and 15 min, 2 h, and 2, 4, 7, 9, 11, 14, 16, and 18 days postchallenge to determine the colonization of *Salmonella* in modified VL media only (controls).

**Fluorescent microscopy.** After isolation from the chemostat culture by selective culturing on brilliant green agar plates, the presence of challenge bacteria, ST-GFP, was confirmed by using a Leica DMLB fluorescent microscope equipped with a 100× oil lens and a FITC filter pack.

**Automated ribotyping.** Ribotyping was performed on pure cultures from the selective media culturing procedure. A RiboPrinter Microbial Characterization System (DuPont Qualicon,

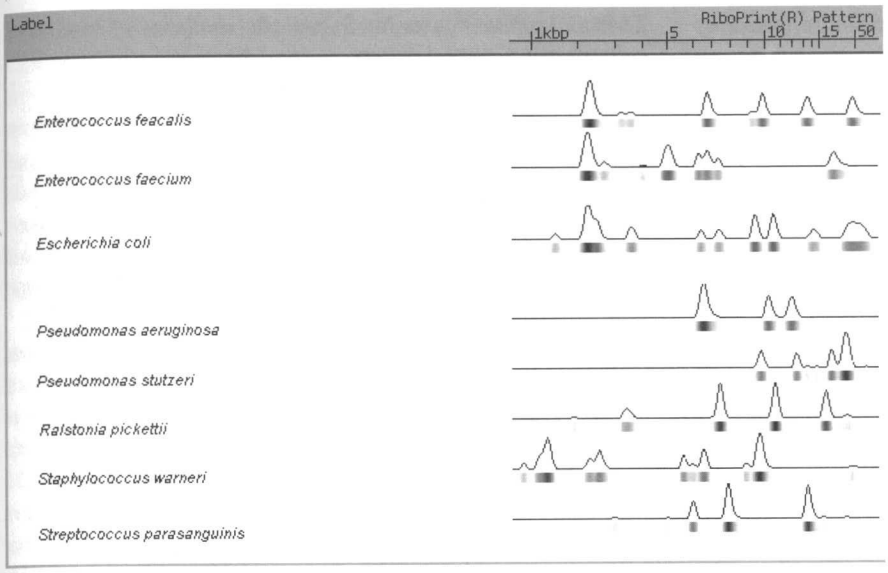


FIGURE 1. *RiboPrint patterns for the eight species of bacteria*—Escherichia coli, Enterococcus faecalis, Enterococcus faecium, Pseudomonas aeruginosa, Pseudomonas stutzeri, Ralstonia pickettii, Staphylococcus warneri, *and* Streptococcus parasanguinis—*isolated from the continuous-flow cultures derived from cecal contents of day-of-hatch chicks.*

Inc., Wilmington, DE) was used for all ribotype analyses. As prescribed by the manufacturer, aerobes were plated onto tryptic soy agar with 5% sheep blood and incubated aerobically at 37°C for 24 h to allow the formation of a bacterial lawn. An aliquot was collected from the lawns by using the collection device provided by the manufacturer (DuPont Qualicon). The bacteria were suspended in a neutral pH buffer (DuPont Qualicon). Strict anaerobes were plated onto *Brucella* blood agar (Anaerobe Systems) and incubated anaerobically at 37°C for 48 h. Single colonies were transferred into tubes containing 9 ml of VL medium and incubated at 37°C for 24 to 48 h. Aliquots (1 ml) of the resulting cultures were centrifuged at 14,000 × *g* for 2 min to pellet the bacteria. Each bacterial pellet was resuspended in sterile phosphate-buffered saline to a turbidity level equivalent to a 6.0 MacFarland standard. All bacterial suspensions were heated at 90°C for 10 min, and combined with two DuPont Qualicon proprietary lytic enzymes. DNA was cleaved by using the restriction endonuclease *Eco*RI (DuPont Qualicon); fragments were separated by gel electrophoresis and analyzed by using a modified Southern hybridization blotting technique. The DNA was hybridized with a labeled rRNA operon probe (DuPont Qualicon) derived from *Escherichia coli,* and the bands were detected by using a chemiluminescent substrate. The resulting image was captured by using a customized charge-coupled device camera, and then electronically transferred to the RiboPrinter Microbial Characterization System database. Each sample lane of data was normalized to a standard marker set. The resulting RiboPrint patterns were compared with a total of 7,348 individual *Eco*RI patterns, consisting of 6,448 in the DuPont database, and an additional 900 in an in-house, custom database of organisms from livestock and poultry sources. An individual ribopattern match of ≥85% of an existing ribopattern was used to characterize each isolate as a specific organism.

TABLE 1. *Eight species isolated from planktonic and biofilm communities, including three species of lactic acid–producing bacteria*

| Species | Community: Planktonic | Community: Biofilm |
|---|---|---|
| Gram positive | | |
| *Enterococcus faecalis*[a] | √ | √ |
| *Enterococcus faecium*[a] | √ | |
| *Staphylococcus warneri*[a] | | √ |
| *Streptococcus parasanguinis* | | √ |
| Gram negative | | |
| *Escherichia coli* | √ | √ |
| *Pseudomonas aeruginosa* | √ | |
| *Pseudomonas stutzeri* | √ | |
| *Ralstonia pickettii* | √ | |

[a] Lactic acid bacterium.

## RESULTS

Determination of the bacterial genera and species by the RiboPrinter involves the analysis of both the number and intensity of bands (DNA fragments) obtained from the restriction enzyme cleavage of each isolate to develop a ribopattern. This system is capable of resolving fragments ranging in size from 1 to 50 kb. The ribopatterns of the eight species isolated from the cultures are shown in Figure 1.

The cecal-derived continuous-flow cultures yielded eight species from six genera. All of the planktonic cultures contained the lactic acid bacteria *Enterococcus faecalis.* One culture, A, also contained *Enterococcus faecium.* All biofilm communities contained the lactic acid bacteria *E. faecalis.* One culture, A, also contained *Streptococcus parasanguinis* in the biofilm. No propionic acid bacteria were isolated. Gram-negative bacteria were predominant in the planktonic communities (four versus two), while gram-positive bacteria made up the bulk of the biofilm communities (three versus one). In general, planktonic communities showed greater diversity than did biofilm communities (Table 1).

*E. faecalis* was the only species found in all of the planktonic communities. The planktonic component from cultures B and C were monocultures of *E. faecalis,* whereas culture A contained *Pseudomonas aeruginosa, E. faecalis, and E. faecium.* Cultures D and E were started from an identical source of combined cecal material, which contained twice the number of birds used to start cultures A,

TABLE 2. *Characterization of planktonic community species by culture by automated ribotyping*

| Species | Culture[a]: A | B | C | D | E |
|---|---|---|---|---|---|
| *Enterococcus faecalis* | 92 | 94 | 96 | 96 | 95 |
| *Enterococcus faecium* | 95 | —[b] | — | — | — |
| *Escherichia coli* | — | — | — | 90 | 90 |
| *Pseudomonas aeruginosa* | 91 | — | — | 93 | 95 |
| *Pseudomonas stutzeri* | — | — | — | 85 | 89 |
| *Ralstonia pickettii* | — | — | — | 87 | 87 |

[a] Values are percent ribotype match for characterization of species.
[b] —, not present in culture.

B, and C. Cultures D and E had identical planktonic communities, but with a community structure more diverse than the other cultures. *Pseudomonas stutzeri, Pseudomonas aeruginosa,* and *Ralstonia pickettii* were only present in planktonic communities and were not found in biofilm communities (Table 2).

*E. faecalis* was the only species found in all of the biofilm communities. In three cultures (B, C, and E), the biofilm consisted of only *E. faecalis.* The biofilm community at level 2 contained the most diversity of species. Culture A had the most diverse biofilm community, consisting of three species from three genera. Further, *S. parasanguinis* and *Staphylococcus warneri* were only found in the biofilm component of this culture. The *S. enterica* serovar Typhimurium that was added to the planktonic portion of cultures A and D was subsequently recovered from these cultures biofilm (Table 3).

ST-GFP was used to challenge bioreactors containing modified VL media only (control) and bioreactors A and D. The presence or absence of ST-GFP within the culture was followed by growth on selective media, enrichment in tetrathionate broth, and confirmation by fluorescence microscopy (Table 4). No other *Salmonella* was found in the cultures. ST-GFP rapidly colonized the control bioreactors containing media only. The cultures obtained a concentration greater than $10^7$ CFU/ml of ST-GFP within 2 days and maintained that concentration for 18 days postinoculation. On challenge of two bioreactors (A and D) with established cecal communities, ST-GFP concentrations quickly dropped over the first 2 days; however, an ST-GFP population remained viable in the cultures at a low level for 18 days.

TABLE 4. *Plate counts for* Salmonella *challenge of two cecal cultures and a control culture*

| Time (days) | Culture: Control, VL only,[a] mean CFU ($\times 10^7$)[b] | Cecal 1A, mean CFU ($\times 10^3$) | Cecal 1D, mean CFU ($\times 10^3$) |
|---|---|---|---|
| 0 | 0.0 ± 0[b] | 0.0 | 0.0 |
| 0.08 | 0.07 ± 0.05 | 5,000.00 | 5,500.0 |
| 0.2 | 0.03 ± 0.01 | 4,000.00 | 8,000.0 |
| 2 | 14.2 ± 3.2 | 0.01 | 0.01 |
| 4 | 3.4 ± 1.2 | 0.01 | 4.0 |
| 7 | 2.7 ± 0.4 | 0.04 | 2.4 |
| 9 | 3.6 ± 1.9 | 0.01 | 4.4 |
| 11 | 1.1 ± 0.3 | 0.10 | 6.3 |
| 14 | 3.8 ± 1.2 | 0.10 | 9.2 |
| 16 | 1.1 ± 0.2 | 0.80 | 4.6 |
| 18 | 1.6 ± 0.7 | 0.01 | 9.1 |

[a] VL-only cultures were challenged with $10^6$ CFU/ml ST-GFP.
[b] The mean ± standard error of three independent control cultures containing VL media only, with no cecal material.

## DISCUSSION

Numerous reports detailing the planktonic microbial communities characterized in cultures from the ceca of adult chickens exist in the literature *(5, 21, 31, 41, 50)*; however, the biofilm portion of these cultures was not characterized. Joseph et al. *(26)* and Trachoo et al. *(47)* report that biofilms are a source of long-term contamination by both *Salmonella* and *Campylobacter jejuni.* Additionally, biofilms are implicated as a factor that enhances the resistance and virulence of *Salmonella (26, 27, 30).* Biofilms can, however, be useful tools in a variety of agricultural situations. Ichida et al. *(25)* demonstrated that the formation

TABLE 3. *Characterization of biofilm community species by level in culture vessel, by automated ribotyping*

| Species | Culture: A % ribotype match | A Vessel level(s)[a] | B % ribotype match | B Vessel level(s) | C % ribotype match | C Vessel level(s) | D % ribotype match | D Vessel level(s) | E % ribotype match | E Vessel level(s) |
|---|---|---|---|---|---|---|---|---|---|---|
| *Enterococcus faecalis* | 91 | 1–5 | 93 | 1–5 | 96 | 1–5 | 96 | 2 | 93 | 2, 4, 5 |
| *Escherichia coli* | —[b] | | — | | — | | 89 | 3 | — | |
| *Staphylococcus warneri* | 95 | 2 | — | | — | | — | | — | |
| *Streptococcus parasanguinis* | 90 | 2 | — | | — | | — | | — | |
| *Salmonella enterica* serovar Typhimurium[c] | | 1–4 | — | | — | | | 4 | | |

[a] Vessel levels: 1, bottom of the chemostat; 2, lowest area of the sidewall; 3, midway up the biofilm; 4, under the biofilm interface ring; 5, biofilm interface ring.
[b] —, not present in culture.
[c] Found in biofilm after addition to planktonic component for challenge; identification was confirmed by fluorescent microscopy.

of a biofilm enhanced the degradation of feathers within a composting system. Zhao et al. *(52)* found that certain biofilms were highly inhibitory to *Listeria monocytogenes*. The work of both Bower and Daeschel *(7)* and Hunt et al. *(24)* suggest that biofilms are an important strategy of microbial survival in both natural and engineered environments, and may have a fundamental role in the development and enhancement of bacterial resistance within agricultural environments.

The role of the biofilm community in the development and maintenance of a model chemostat cultures needs to be examined. This study examined bacterial communities derived from the ceca of day-of-hatch chickens and characterized the planktonic communities in combination with the biofilm communities. To our knowledge, this is the first report that characterizes both the planktonic and biofilm communities of continuous-flow cultures derived from the cecal microflora by automated ribotyping. It is recognized that the cecal microflora can be significantly influenced by environmental factors; therefore, the data presented here should be regarded as case specific.

Of the five cultures characterized in this study, three cultures were started from independent sets of five chicks and had only one bacterial species, *E. faecalis*, in common. The remaining two cultures were started from replicate aliquots of the same material obtained by combining the cecal contents of 10 chicks. The community composition was more complex in these combined cultures (D and E), which represented a compilation of twofold as many birds. Interestingly, the planktonic communities within the replicate cultures were identical, whereas the biofilm communities were different. The data show that unlike data of Bradshaw et al. *(8)*, the biofilms in these systems were not a direct reflection of the surrounding planktonic community. *E. faecalis* was the only species common to both communities in all cultures. Two cultures (A and E) had several species that segregated into either the biofilm or the planktonic portion only. Further, culture A was the only culture in which the biofilm community was more diverse than was the planktonic community. Cultures D and E both contained *P. stutzeri* and *R. pickettii*. While neither of these is normally found in healthy chicks, Mead *(28)* points out that occasionally, transient organisms picked up from the environment appear in the microbial population of the GIT.

One possible explanation for these differences between the planktonic and biofilm communities is the relatively long residence time in rich media selected for fast growers in the planktonic component of the chemostat, whereas the slow growers were sequestered into the biofilm. Additionally, adjustments in population density, diversity, and adhesion occurring during the culturing period play a part in this observation. Further, the differences among bacterial species found at the different levels of biofilm can be attributed to many factors. According to Davey and O'Toole *(12)*, most biofilms exhibit some level of heterogeneity and are characterized by patches of cell aggregates, not monolayers. Moreover, the development and deposition of the various species can be influenced by several environmental parameters such as surface and interface properties, the composition of the microbial community, and hydrodynamics of the vessel *(12)*. One constant factor was that the introduced ST-GFP was isolated from all of the culture biofilms. This sequestration of the *Salmonella* could be an influential factor in the cultures inability to withstand invasion by the introduced ST-GFP.

The early posthatch stage is critical for establishment of the gut microbial community, and chicks are notably more vulnerable to *Salmonella* infection at this stage *(37, 45)*. The gastrointestinal ecosystem contains habitat and niches for microbe colonization, which until birth is relatively sterile. After hatch, chicks pick up a variety of microbial inhabitants derived from their surroundings, and there is a succession of colonization until all habitats within the GIT are occupied by climax communities *(29, 40)*. Chicks in production facilities may be particularly at risk due to a delayed development of their intestinal flora because of sanitized conditions and restricted access between generations. A nonindigenous microbe such as *Salmonella* is usually transitory when introduced into a healthy, well-functioning adult gut, in which the available niches are occupied by climax communities of indigenous microbes. However, such a microbe may establish a foothold in a habitat not yet occupied, or one vacated by indigenous inhabitants after some perturbation. The economic impact of subsequent human illness from foodborne pathogens in processed foodstuffs can be substantial *(38)*.

The susceptibility of the host to invasion and subsequent long-term retention of a pathogen changes with age and with the volume and complexity of indigenous GIT microbes. In this study, we evaluated the success of colonization by *Salmonella* within the established day-of-hatch ceca culture. The cultures contained few microbes in comparison to cultures initiated from older birds *(4, 11, 39)* and were unable to completely restrict colonization by *Salmonella*, indicating that the combination of microbes in these cultures was perhaps not complex enough. However, the level of *Salmonella* colonization was reduced in comparison to controls, presumably due to the influence of the resident bacteria.

Researchers have shown that volatile fatty acids produced by lactic and propionic acid bacteria within the ceca of chicken play a key role in providing protection from *Salmonella* invasion. Corrier et al. *(11)* showed a direct correlation between elevated levels of propionic acid and decreased numbers of *Salmonella*. van der Wielen et al. *(49)* demonstrated that the bacteriostatic effect of volatile fatty acids is one of the mechanisms by which intestinal microflora reduce or eliminate *Salmonella*. Lower levels of these same volatile fatty acids were not capable of eliminating *Salmonella* completely, but they were able to lower its biomass *(48)*. The presence of a small number of lactic acid bacteria within the ceca of day-of-hatch chicks could partially explain why we observed a reduction in *Salmonella* numbers but never completely cleared the *Salmonella* from the cultures. Further, the difference in *Salmonella* numbers observed between cultures A and D could well be related to the fact that culture A contained three lactic acid bacteria, while culture D contained only one. While we did

not isolate propionic acid bacteria from day-of-hatch ceca, the presence of lactic acid bacteria in the ceca in young birds may lead to favorable conditions for the colonization of the GIT by propionic bacteria at a later age *(10)*.

The colonization process within the GIT is influenced by age, the environment, and by changes exerted by the makeup of the colonizers themselves. The complexity of GIT bacteria in day-of-hatch birds is limited in comparison to adult birds; therefore, characterization of the composition of microbes is more clear-cut. This study is the first of several that evaluate the composition of microbial communities in both the planktonic and biofilm components of in vitro cultures established from chicks of different ages. Further research is also needed to ascertain the influence of the sequestered bacterial communities within the biofilm of continuous-flow culture systems on the planktonic portion of the culture, which is the portion harvested for competitive exclusion.

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